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# SENSITIVITY OF SPORES OF EIGHT *BACILLUS CEREUS* STRAINS TO PRESSURE-INDUCED GERMINATION BY MODERATE HYDROSTATIC PRESSURE, TIME AND TEMPERATURE

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The spores of eight *Bacillus cereus* strains were pressurized at 138 to 483 MPa for 5 to 20 min at 25° to 70°C in order to determine the sensitive and the resistant strains to pressure-induced germination. The most sensitive strain was *B. cereus* QMB 476, while the most resistant strain was *B. cereus* ATCC 11778. Pressurization at 25°C induced spores to germinate ranged from 2.6 to 5.0 log cycles. An addition of 1.3 to 1.9 log cycle increased in pressure-induced germination of *B. cereus*, when spores were pressurized at 50°C. Pressurization at 483 MPa for 20 min at 70°C, spores of *B. cereus* QMB 476 were inactivate from 11.9 to 4.0 log cycles (7.9 log reductions) and spores of *B. cereus* ATCC 11778 were killed from 9.2 to 3.1 log cycles, 6.1 log reductions. The survivors were destroyed, when spores were pressurized in the presence of bacteriocin-based preservatives containing 100 µg/ml of lysozyme and 500 µg/ml of Na-EDTA.

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| BACILLUS CEREUS | HYDROSTATIC PRESSURE | TEMPERATURE | PACKAGING |
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## PREFACE

This study was conducted from July 2002 through June 2003 by Mssrs. Norasak Kalchayanand and Bibek Ray, University of Wyoming, under supervision of Mssrs. Anthony Sikes and Patrick Dunne, U.S. Army Soldier Systems Center, Natick, MA 01760. The work was funded under the project titled "Pressure Inactivation Parameters of Foodborne Pathogenic *Bacillus cereus* Spores," Contract number DAAN02-98-C-4010.

Mssrs. Kalchayanand's and Ray's research was designed to ascertain answers to the following questions: (1) are spores of different strains of *B. cereus* sensitive to hydrostatic pressure induction to germination; (2) will a combination of moderate pressure and temperature inactivate a high level of spores of *B. cereus*, and since both *B. cereus* and *Bacillus anthracis* share many common physiological characteristics, will they, *B. anthracis*, exhibit a similar responds to hydrostatic pressure-induced germination, and (3) does hydrostatic pressure in combination with bacteriocin-based preservatives enhance viability loss of *B. cereus* spores?

# SENSITIVITY OF SPORES OF EIGHT *BACILLUS CEREUS* STRAINS TO PRESSURE-INDUCED GERMINATION BY MODERATE HYDROSTATIC PRESSURE, TIME AND TEMPERATURE

## Introduction

Unlike vegetative cells, the inactivation of bacterial spores is still a major concern of the application of hydrostatic pressure for the preservation of foods. The mode of action of pressure on bacterial spores is not clear (Smelt, 1998). However, bacterial spores can be destroyed directly by pressure higher than 1000 MPa. A combination of very high pressure and high temperature was also used to destroy many spores of *Bacillus* and *Clostridium* species (Farkas and Hoover, 2000; Ray et al., 2001; Ray 2002). This drastic treatment has adversely affected the quality of food and has limited commercial application. However, bacterial spores are sensitive to pressures between 50 and 300 MPa (Sale et al., 1970; Wuytack et al., 1998). It has been observed for the spores of various *Bacillus* species that the inactivation was more efficient at moderate (200 to 500 MPa) rather than at higher (>500 MPa) pressures (Wuytack et al., 1998). It is generally agreed that at such pressures, spores can be induced to germinate followed by the death of the germinated spores under those pressures (Clouston and Wills, 1969; Gould and Sale, 1970; Sale et al., 1970; Sojka and Ludwig, 1994).

*Bacillus cereus* is ubiquitous in nature and its spores may be present normally at low levels in food ($<10^2$ cfu/g; Forsythe, 2000). *B. cereus* is recognized as a causative agent of food poisoning. Foods incriminated in the past outbreaks of *B. cereus* poisoning include cereal dishes containing corn and corn starch, potatoes, cold soups, sauces,

puddings, meats, cooked vegetables, ice cream and fresh and powdered milk (Doyle, 1988; Snyder and Poland, 1991). Very limited information on pressure induction and inactivation of *B. cereus* spores exists. The objectives of this study are to determine the sensitivity of pressure-induced germination among the spores of B. *cereus* strains to hydrostatic pressure, pressurization time and temperature, and to develop the pressurization parameters for the effective inactivation of *B. cereus* spores at a high level. In addition, as *B. cereus* and *B. anthracis* have many common physiological characteristics, these results may have important implications on the pressure-inactivation of *B. anthracis* spores, which has become a major safety concern.

## Materials and Methods

### Bacterial strains

Eight strains of *Bacillus cereus* spores were used in this study. Four strains were obtained from Anthony Sikes, US Army Research, Development and Engineering Command, Natick, MA, and four were from U. of Wyoming (UW) Food Microbiology culture collection (Table 1). Cultures were grown aerobically in nutrient broth with 500 units per ml of polymyxin B sulfate at 30°C for 48h. Each strain was streaked on antibiotic assay medium (AAMS; Cook and Brown, 1964) supplemented with 500 units/ml of polymyxin B sulfate and incubated at 30°C for 24 to 48h. A single colony of each strain was transferred to AAMS broth without antibiotic and incubated for 16 to 18h at 30°C. The cells were harvested by centrifugation at 8000xg for 10 min and followed with the API 50 CHB identification scheme (bioMérieux, Hazelwood, MO). The biochemical characteristics were confirmed with the computer database provided by bioMérieux (bioMérieux, Hazelwood, MO) and stock cultures were made and stored at –40°C.

Table 1. *Bacillus cereus* sources and description

| Strain | Source | Description |
|---|---|---|
| *B. cereus* 16 | US Army | Isolated form salmon steak; Nisin resistant at 250 ppm. |
| *B. cereus* 19 | US Army | Isolated from salmon steak treated with allyl isothio-cyanate. |
| *B. cereus* QMB 476 | US Army | From QM stock. |
| *B. cereus* SLR 779 | UW; Fd Micro. | Isolated from tomato paste |
| *B. cereus* QMB 1565 | US Army | From QM stock. |
| *B. cereus* ATCC 10876 | ATCC | |
| *B. cereus* ATCC 11778 | ATCC | |
| *B. cereus* 13103 | UW; Fd Micro. | |

Bacteriocin-producing *Pediococcus acidilactici* LB 42-923 and *Lactococcus lactis* ATCC 11454 (UW Food Microbiology culture collection) were grown in trypticase glucose yeast extract (TGE) at 37° and 30°C, respectively (Bhunia et al., 1988; Yang et al., 1992).

**Preparation of *B. cereus* spores**

All the cultures, except *B. cereus* ATCC 11778, were transferred to Kim-Goepfert (KG; Leininger, 1976) broth without egg yolk emulsion and polymyxin B sulfate and agitated at 3000 rpm for 72 h at 30°C. After 72 h of incubation, cultures were observed under phase contrast microscope for the presence of spores.

A large number of spores from *B. cereus ATCC* 11778 were successfully obtained on nutrient agar (Difco, Detroit, MI) supplemented with 50 mg/L of $MnSO_4 \cdot H_2O$ (Sigma, St. Louis, MO). All spores were harvested and washed six times with sterile deionized water by centrifugation at 8000xg for 10 min at 4°C. Spores were resuspended with sterile deionized water and stored at 4°C. The spore concentration was determined by heat-activation at 80°C for 10 min, serially diluted with 0.1% peptone solution and plated with AAM supplemented with 0.1% soluble starch and without antibiotic. Plates were incubated at 30°C for 48h before counting the colonies.

**Preparation of antimicrobial compounds**

The two bacteriocins, pediocin AcH and nisin A, were obtained from the producer strains *Pediococcus acidilactici* LB 42-932 and *Lactococcus lactis* ATCC 11454, respectively. For pediocin AcH production, *P. acidilactici* LB 42-923 was grown in TGE at 37°C for 16 to 18 h. *L. lactis* ATCC 11454 was grown in TGE using fermenter (Biostat M; B. Braun, Allentown, PA) at 30°C for 16 to 18 h at a constant pH of 5.8 for nisin A production. Both pediocin AcH and nisin A were partially purified by the adsorption and desorption methods as described by Yang et al., 1992. Both bacteriocins were freeze-dried, assayed for their potencies as activity unit (AU)/g of dried materials and stored at –40°C according to Bhunia et al., 1988, Biswas et al., 1991 and Young et al., 1992. Before using, the dried materials were dissolved in sterile deionized water, sterilized by membrane filtration (0.22 μm; Pall Gelman, Ann Arbor, MI) and assayed for AU/ml.

The bacteriocin-based preservatives (BP and $BP_Y$) were prepared using the bacteriocin solutions as follows: BP was prepared by mixing pediocin and nisin of equal AU/ml at 3:7 ratio; $BP_Y$ was prepared by using BP supplemented with 100 μg/ml of lysozyme (Sigma, St. Louis, MO) and 500 μg/ml of Na-EDTA (Sigma, St. Louis, MO). Both BP and $BP_Y$ were used at either 500 or 3,000 AU/ml final concentration.

**Hydrostatic pressurization**

The vials containing spore suspension with or without biopreservative based antimicrobial compounds were subjected to an initial heat treatment either at 27.5°, 53.5°, 63.5° or 73.5°C for 5 min to ensure that the temperature at the middle of vials did reach 25°, 50°, 60° or 70°C before placing into the preheated pressurization chamber (Engineered Pressure System, Wilmington, MA) containing a mixture of water and 5% soluble oil (Hydrolubric 2; Houghton International, Valley Forge, PA). Controls were not subjected to hydrostatic pressure treatment. The vials were placed inside the chamber and held for 2 min to equilibrate the temperature before pressurization from 138 to 483 MPa for 5, 10 or 20 min. The pressurized vials were cooled immediately in an ice-bath for 5 min and stored at 4°C for 24h before enumeration for colony forming unit (CFU).

**Enumeration of total and pressure-induced spores**

The total spore concentrations were determined by plating following heat-activation at 80°C for 10 min. The heat killed the vegetative cells present in the preparation.

The heat-activated spore suspension of each vial was serially diluted in 0.1% peptone solution and was plated in duplicate. AAMS with or without bio-preservative-based antimicrobial compounds was used to enumerate. The plates were incubated aerobically at 30°C for 48h and CFU from a dilution that gave CFU between 25 to 250 colonies per plate were counted.

To determine germinated spores in a population, the pressurized spore suspensions were given a heat treatment (80°C for 10 min) to destroy the pressure-induced germinated spores and plated as described above to enumerate the uninduced post-pressurized spores. The differences in counts between the total spore concentrations and uninduced post-pressurized spores gave the numbers of pressured-induced spores.

**Statistical analysis**

A randomized complete block design of the pooled data was used with growth of spores measured as $\log_{10}$ CFU/ml for analyses of variance (ANOVA). Differences in means were determined by using Duncan's Multiple Range Test methods (SAS, 1985).

# RESULTS AND DISCUSSION

## Identification of *B. cereus* strains

All tested strains were confirmed with the computer database provided by bioMérieux (bioMérieux, Hazelwood, MO), as well as biochemical characteristics from Bergey's Manual of Systematic Bacteriology (Sneath, P.H.A. et al., 1986) to be *B. cereus*. All strains produced acid form glucose, maltose, trehalose and hydrolyzed esculine compared to the biochemical reactions of this organism in Bergey's Manual (Table 2). Ribose and fructose were used as a carbon source among the tested strains. *B. cereus* 19, ATCC 11778 and 13103 did not produce acid from glycerol. *B. cereus* QMB476, QMB1565, ATCC 10876 and 13103 did not use saccharose as a carbon source. *B. cereus* SLR 779 isolated from tomato paste and *B. cereus* 13103 did not produce acid from salicine. Under phrase contrast microscope, all strains showed ellipsoidal spores at the middle of the cells.

## Pressure-induced germination of *B. cereus* strains by the combined effect of pressure, time and temperature.

The spore suspensions of the eight strains of *B. cereus* were exposed to different combinations of pressure ranging between 138 and 483 MPa for 5 to 20 min at 25° or 50°C. Pressurization at 138 MPa for 20 min at 50°C resulted in pressure-induced germination of spores of *B. cereus* 16, QMB 476 and QMB 1565. The pressure-induced germination results at 20° and 50°C are given in Table 3 and 4, respectively. Pressure-induced germination ranged from 1.65 to 6.08 log cycles, when spores were pressurized at 25°C for 5 to 20 min.

Increasing the pressure from 138 to 483 MPa at 25°C did not always increase the number of pressure-induced spores. Gould and Sale (1970) and Wuytack et al. (1998) also reported this phenomenon. In this study, a number of pressure-induced spores of *B. cereus* QMB476, QMB1565 and ATCC 10876 increased with increasing pressure from 138 to 483 MPa. Increasing time of pressurization from 5 to 20 min resulted in increasing the number of pressure-induced spores of all tested strains at each level of pressure. The number of pressure-induced spores of *B. cereus* T increased, when those spores were pressurized at 51 MPa for 5 to 30 min as reported by Gould and Sale (1970). When spores were pressurized at 50°C for 5 to 20 min, the number of pressure-induced germinated spores ranged from 2.88 to 7.59 log cycles. It was also observed that an additional 1.23 to 1.51 log cycle increase occurred when spores were pressurized at 50°C. Further, the number of pressure-induced spores increased with increasing pressure of 138 to 483 MPa. This suggested that pressure-induced germination of spores of *B. cereus* was temperature dependent. Gould and Sale (1970) found that the amount of pressure-induced spores of *B. coagulans*, *B. subtilis* and *B. cereus* T were higher, when those spores were pressurized at 50° to 60°C. Similar to pressurization at 25°C, the number of pressure-induced spores increased with increasing pressurization time from 5 to 20 min. In all tested strains, pressurization at 50°C had a higher induction effect than at 25°C. In this study, the optimum conditions for pressure-induction of spores of *B. cereus* were not clear. Differences in germination level of *Bacillus* spores as a function of pressure and temperature have been reported by other investigators (Gould and Sale, 1970; Gould, 1973; Nakayama et al., 1996; Wuytack et al., 1998; Okazaki et al., 2000; Farkas and Hoover, 2000).

Table 2. Biochemical characteristics of *B. cereus* strains relative to *B. cereus* from Bergey's Manual

| Strain | Characteristics[b] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Gly | Rib | Glu | Fruc | Esc | Sal | Malt | Sacc | Treh | Spores shape/spore position |
| *B. cereus*[a] | + | | + | | + | + | + | + | + | Ellipsoidal/Central |
| *B. cereus* 16 | + | + | + | + | + | + | + | + | + | Ellipsoidal/Central |
| *B. cereus* 19 | - | + | + | + | + | + | + | + | + | Ellipsoidal/Central |
| *B. cereus* QMB476 | + | + | + | + | + | + | + | - | + | Ellipsoidal/Central |
| *B. cereus* SLR 779 | + | + | + | + | + | - | + | + | + | Ellipsoidal/Central |
| *B. cereus* QMB1565 | + | + | + | + | + | + | + | - | + | Ellipsoidal/Central |
| *B. cereus* ATCC 10876 | + | + | + | + | + | + | + | - | + | Ellipsoidal/Central |
| *B. cereus* ATCC 11778 | - | + | + | + | + | + | + | + | + | Ellipsoidal/Central |
| *B. cereus* 13103 | - | + | + | + | + | - | + | - | + | Ellipsoidal/Central |

[a] Strain reported from Bergey's Manual of Systematic Bacteriology Vol. 2, 1986.

[b] Gly, glycerol; Rib, ribose; Glu, glucose; Fruc, fructose; Esc, esculine; Sal, salicine; Malt, maltose; Sacc, saccharose; and Treh, trehalose

Table 3. Pressure-induced spores of *B. cereus* strains following pressurization at 25°C.

| Strain # | Time (min) | $\log_{10}$ induction following pressurization at (MPa) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 138 | 207 | 276 | 345 | 414 | 483 |
| 16 | 5 | 5.54 | 2.89 | 2.92 | 2.36 | 2.19 | 2.08 | 2.15 |
| | 10 | | 2.94 | 2.89 | 2.30 | 2.39 | 2.56 | 2.52 |
| | 20 | | 3.15 | 2.61 | 3.00 | 2.68 | 2.42 | 2.56 |
| 19 | 5 | 7.60 | 3.91 | 4.12 | 3.18 | 3.34 | 3.54 | 3.31 |
| | 10 | | 4.00 | 4.06 | 3.31 | 4.00 | 4.06 | 4.20 |
| | 20 | | 4.68 | 4.62 | 4.73 | 5.01 | 4.54 | 4.40 |
| 476 | 5 | 6.08 | 4.21 | 4.43 | 4.38 | 4.00 | 4.54 | 4.69 |
| | 10 | | 4.68 | 4.93 | 4.69 | 4.60 | 4.60 | 4.93 |
| | 20 | | 5.58 | 5.93 | 5.63 | 6.08 | 6.08 | 6.08 |
| 779 | 5 | 7.19 | 3.39 | 3.42 | 3.91 | 3.84 | 2.90 | 2.80 |
| | 10 | | 4.00 | 4.04 | 3.98 | 3.93 | 3.76 | 3.89 |
| | 20 | | 5.01 | 5.17 | 6.04 | 5.65 | 5.02 | 5.19 |
| 1565 | 5 | 6.30 | 1.65 | 1.65 | 2.49 | 2.46 | 2.38 | 2.38 |
| | 10 | | 2.30 | 2.40 | 3.70 | 3.35 | 3.18 | 3.18 |
| | 20 | | 3.85 | 5.00 | 4.91 | 4.65 | 4.02 | 4.21 |
| 10876 | 5 | 6.84 | 2.56 | 2.69 | 2.66 | 3.66 | 3.78 | 4.00 |
| | 10 | | 2.94 | 3.71 | 3.72 | 3.80 | 3.94 | 4.14 |
| | 20 | | 3.19 | 3.74 | 3.86 | 3.86 | 4.00 | 4.15 |
| 11778 | 5 | 6.30 | 2.56 | 2.65 | 2.61 | 2.65 | 2.49 | 2.28 |
| | 10 | | 2.76 | 2.76 | 2.70 | 2.55 | 2.70 | 2.52 |
| | 20 | | 2.82 | 3.14 | 2.93 | 3.12 | 3.04 | 2.98 |
| 13103 | 5 | 6.90 | 3.80 | 3.21 | 3.06 | 2.64 | 2.66 | 2.21 |
| | 10 | | 4.30 | 4.30 | 3.62 | 3.63 | 3.63 | 3.63 |
| | 20 | | 4.47 | 4.98 | 4.86 | 4.77 | 4.86 | 5.00 |

Table 4. Pressure-induced of spores of *B. cereus* strains following pressurization at 50°C.

| Strain # | Time (min) | $\log_{10}$ induction following pressurization at (MPa) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 138 | 207 | 276 | 345 | 414 | 483 |
| 16 | 5 | 5.84 | 3.71 | 3.82 | 4.36 | 4.36 | 4.54 | 4.54 |
| | 10 | | 4.69 | 4.99 | 5.19 | 4.69 | 4.84 | 4.84 |
| | 20 | | 5.84 | 5.84 | 5.84 | 5.84 | 5.84 | 5.84 |
| 19 | 5 | 6.92 | 4.22 | 4.27 | 4.86 | 5.27 | 4.90 | 4.97 |
| | 10 | | 4.59 | 4.56 | 5.77 | 5.62 | 5.22 | 5.38 |
| | 20 | | 5.44 | 5.92 | 6.47 | 6.77 | 6.92 | 6.92 |
| 476 | 5 | 6.54 | 5.89 | 5.74 | 5.69 | 6.54 | 6.54 | 6.39 |
| | 10 | | 6.54 | 6.54 | 6.39 | 6.54 | 6.39 | 6.54 |
| | 20 | | 6.54 | 6.54 | 6.54 | 6.54 | 6.54 | 6.54 |
| 779 | 5 | 6.16 | 3.77 | 4.01 | 4.36 | 4.46 | 4.31 | 4.21 |
| | 10 | | 5.31 | 5.26 | 5.46 | 5.77 | 6.16 | 6.01 |
| | 20 | | 6.16 | 6.16 | 6.16 | 6.16 | 6.16 | 6.16 |
| 1565 | 5 | 6.40 | 3.86 | 4.01 | 4.56 | 4.45 | 4.36 | 4.70 |
| | 10 | | 4.43 | 5.55 | 5.45 | 5.10 | 5.40 | 5.70 |
| | 20 | | 5.70 | 6.40 | 6.40 | 6.40 | 6.40 | 6.40 |
| 10876 | 5 | 6.72 | 2.88 | 2.98 | 3.12 | 4.18 | 4.36 | 4.33 |
| | 10 | | 4.07 | 4.07 | 4.24 | 4.24 | 4.24 | 4.40 |
| | 20 | | 4.70 | 4.72 | 5.33 | 5.27 | 6.72 | 6.72 |
| 11778 | 5 | 6.00 | 3.26 | 3.52 | 3.70 | 3.92 | 4.35 | 3.46 |
| | 10 | | 3.46 | 3.98 | 4.40 | 4.85 | 4.70 | 4.46 |
| | 20 | | 5.00 | 5.15 | 6.00 | 6.00 | 6.00 | 5.30 |
| 13103 | 5 | 7.59 | 4.72 | 5.26 | 5.70 | 5.67 | 5.75 | 7.09 |
| | 10 | | 4.94 | 6.20 | 6.20 | 6.74 | 6.59 | **7.59** |
| | 20 | | 6.44 | 6.59 | 6.44 | 7.05 | 6.89 | 7.59 |

**Sensitivities and resistances of spores of *B. cereus* strains to pressure-induced germination**

Pressurization at 25° or 50°C was used to select the sensitive or resistant strains. From the results in Table 1 and 2, the data were pooled for each strain from 5 to 20 min of pressurization and for each pressure. The sensitive and resistant strains were determined through the main effect of pressure to induce spores to germinate at 25° and 50°C, respectively.

The results are presented in Table 5. Among 8 strains of *B. cereus*, hydrostatic pressure-induced spore germination ranged from 2.6 to 5.0 and from 4.5 to 6.3 log cycles, when spores were pressurized at 25° and 50°C, respectively. At 25°C, the *B. cereus* QMB 476 was the most sensitive strain which had the highest log induction among the tested strains ($P \leq 0.05$). At 50°C, both strains of *B. cereus* ATCC 10876 and 11778 had the lowest log induction among the tested strains ($P \leq 0.05$). Therefore, the strains ATCC 10876 and 11778 were pressurized at 138 to 483 MPa for 5 to 20 min at 60° or 70°C to determine which strain was the most resistant to pressure induction. Pressurization at 60°C did not differentiate pressure-induced resistance between *B. cereus* ATCC 10876 and *B.* cereus ATCC 11778 (Table 6). At 70°C, however, the *B. cereus* ATCC 11778 demonstrated a significantly ($P \leq 0.05$) lower log induction number than strain ATCC 10876 and was the most resistant to pressure-induced germination among all of the tested *B. cereus* strains.

Table 5. Influence of hydrostatic pressure on induction to germination of *Bacillus* spores.

| Bacteria strain | $\log_{10}$ induction[a] after pressurized at | |
|---|---|---|
| | 25°C | 50°C |
| *B. cereus* 16 | 2.6[b] | 5.0[bc] |
| *B. cereus* 19 | 4.0[c] | 5.4[c] |
| *B. cereus* QMB 476 | 5.0[d] | 6.2[d] |
| *B. cereus* SLR 779 | 4.2[c] | 5.3[c] |
| *B. cereus* QMB 1565 | 3.2[e] | 5.3[c] |
| *B. cereus* ATCC 10876 | 3.6[f] | 4.5[b] |
| *B. cereus* ATCC 11778 | 2.7[b] | 4.5[b] |
| *B. cereus* 13103 | 3.9[cf] | 6.3[d] |

[a] Pooled data of all pressures and times of pressurization at each temperature
[b-f] Means in the same column bearing a common letter do not differ significantly at $P \leq 0.05$

Table 6. Pressure induced germination of *B. cereus* spores at 60° and 70°C

| Bacterial strain | Temp (°C) | $log_{10}$ induction[a] following pressurization at (MPa) | | | | | | Average $log_{10}$ induction |
|---|---|---|---|---|---|---|---|---|
| | | 138 | 207 | 276 | 345 | 414 | 483 | |
| *B. cereus* 10876 | 60 | 6.15 | 6.40 | 6.76 | 7.13 | 7.10 | 7.24 | 6.80[b] |
| *B. cereus* 11778 | 60 | 5.89 | 6.21 | 6.48 | 6.78 | 6.80 | 6.89 | 6.51[b] |
| *B. cereus* 10876 | 70 | 6.34 | 6.71 | 7.08 | 7.30 | 7.33 | 7.50 | 7.04[c] |
| *B. cereus* 11778 | 70 | 5.60 | 5.84 | 6.20 | 6.58 | 6.71 | 6.72 | 6.28[d] |

[a] Pooled data of pressurization times of 5, 10 and 20 min

[b-d] Means in the same pressurization temperature bearing a common letter do not differ significantly at $P \leq 0.05$

**Viability loss by pressurization in the presence of bacteriocin-based antimicrobial compounds**

The selected strains, one sensitive and one resistant to pressure-induction, were used in this study. The results are presented in Table 7. With pressurization alone, there were some survivors even the most sensitive strain (*B. cereus* QMB 476). Pressurization at 483 MPa at 70°C reduced the number of spores of *B. cereus* QMB 476 from 11.9 to 4.0 log cycles (7.9 log reductions) in 20 min. Similarly, *B. cereus* ATCC 11778 was reduced 6.1 log (from 9.2 to 3.1) in 20 min at the same pressure and temperature. To estimate the beneficial effect of adding bacteriocin-based preservatives, the spore suspensions with BP or $BP_Y$ (3,000 AU/ml) were pressurized and enumerated for viable spores in the AAM agar with or without antimicrobial compounds (500 AU/ml). Pressurization of each spore suspension in the presence of BP did not reduce the number of survivors. This indicates that pressure-induced germinated spores were not sensitive to BP. However, by plating the pressurized spore suspension in the medium containing antimicrobial compounds, the number of survivors was reduced from 4.0 log to 2.7 log cycles in *B. cereus* QMB 476. *B.cereus* ATCC 11778 probably was not sensitive to BP due to only 0.1 log cycle difference in both media. The viability loss increased when $BP_Y$ was include during pressurization. There were no survivors found from either strain when pressurized in the presence of $BP_Y$ or enumerated with medium containing $BP_Y$ . This shows that $BP_Y$ was more effective than BP in destroying the outgrowth of pressure-induced spores of *B. cereus*. The combination of lysozyme and EDTA resulted in a unique synergistic action with pressure to inactivate spores of *B.* cereus.

Table 7. Survivors of the spores of *B. cereus* following pressurization at 483 MPa at 70°C for 20 min in the absence or presence of bacteriocin-based antimicrobial compounds.

| Bacterial strain | Treatments[a] and $log_{10}$ CFU/ml | | | | | |
|---|---|---|---|---|---|---|
| | Ctrl | HP | HP+BP | | HP+$BP_Y$ | |
| | | | Agar-[b] | Agar+[c] | Agar-[b] | Agar+[c] |
| *B. cereus* QMB 476 | 11.9 | 4.0 | 4.0 | 2.7 | ND[d] | ND |
| *B. cereus* ATCC 11778 | 9.2 | 3.1 | 3.1 | 3.0 | ND | ND |

[a] Ctrl: unpressurized control; HP: pressurized, heat treated at 80°C for 0 min and enumerated for CFU/ml; HP+BP: pressurized in the presence of 3,000 AU/ml of bacteriocin-based preservative; HP+$BP_Y$: pressurized in the presence of 3,000 AU/ml of BP supplemented with 100 μg/ml of lysozyme and 500 μg/ml of Na-EDTA.

[b] Pressurized spore suspensions in the presence of one of the bacteriocin-based preservatives were heated and enumerated.

[c] Pressurized spore suspensions from "b" were plated in the AAM-agar containing either BP or $BP_Y$ (500 AU/ml).

[d] ND: no CFU was detected by plating 2 ml of undiluted spore suspension.

# CONCLUSIONS

1. Variation in sensitivity to pressure-induced germination of spores was found among the strains of *B. cereus*. The sensitivity to pressure-induction was depended on magnitude of pressure, pressurization time, temperature and also strains within that species.
2. The most sensitive and resistant strains among the 8 strains tested were *B. cereus* strain QMB 476 and strain ATCC 11778, respectively.
3. There were no specific conditions necessary for pressure-induced germination of spores of *B. cereus*, when using a medium range hydrostatic pressure (>100 MPa to 500 MPa). However, studies have showed that pressure-induced germination is both time and temperature dependent. It seems that spores of *B. cereus* were more sensitive to pressure-induced germination when spores were pressurized for a longer time and at a higher temperatures.
4. Bacteriocin-based preservatives, $BP_Y$, can be used to control *B.* cereus spores during pressurization or post-pressurization.

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